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FLOODING CONSTRAINTS ON TREE (*TAXODIUM DISTICHUM*) AND HERB GROWTH RESPONSES TO ELEVATED CO_2

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Abstract: Elevated CO_2 generally stimulates C_3-type photosynthesis, but it is unclear how an increase in CO_2 assimilation will interact with other factors that influence plant growth. In wetlands, the response of plants to elevated CO_2 will interact with soil saturation, particularly in forested wetlands where soil saturation is a strong regulator of plant productivity. We performed a four-month experiment to determine whether elevated CO_2 and flooding interact to influence the growth of a flood-tolerant tree (*Taxodium distichum*) and a flood-tolerant herbaceous emergent macrophyte (*Orontium aquaticum*). Seedlings were grown in glasshouses at two CO_2 levels (350 and 700 $\mu L\ L^{-1}$) crossed with two water depths (5 cm above and $\geq$5 cm below the soil surface). We hypothesized that elevated CO_2 would increase photosynthesis regardless of water depth and species; however, we also expected flooding to prevent elevated CO_2 from increasing the growth of the tree species due to O_2 limitation or other physiological stresses associated with reduced soil environments. We found that elevated CO_2 increased whole-plant photosynthesis in both species regardless of the flooding treatment. For *T. distichum*, this higher photosynthetic rate resulted in greater biomass only in the non-flooded treatment. This result suggests that some factor related to flooding constrained the biomass response of the flooded woody plants to elevated CO_2. In contrast, elevated CO_2 increased *O. aquaticum* biomass regardless of the flooding regime, perhaps because it occurs in wetter landscape positions than *T. distichum* and is less sensitive to flooding. We conclude that flooding may limit plant growth responses to elevated CO_2, particularly in woody plant species.

Key Words: elevated CO_2, emergent aquatic macrophyte, photosynthesis, wetland, *Taxodium*, global change, flooding

INTRODUCTION

Plants are growing in an atmosphere that is enriched in CO_2 by 33% over pre-industrial levels, and CO_2 concentrations will continue to increase throughout the 21st century. Elevated CO_2 generally stimulates C_3-type photosynthesis (Curtis 1996), but it is uncertain how enhanced photosynthesis will interact with a variety of other factors that influence plant growth (Körner 2000). Responses of upland plants to elevated CO_2 can be constrained by nutrient availability (Zak et al. 1999), water availability (Loustau et al. 2001, Hungate et al. 2002), and tropospheric ozone concentration (Dickson et al. 1998); very little is known about factors that interact with elevated CO_2 in wetlands. Interactions with elevated CO_2 must be addressed because it is clear that elevated CO_2 will be accompanied by global warming and a variety of other environmental changes (Ramaswamy et al. 2001), and these effects are often non-additive (Pendall et al. 2004).

Relatively few studies have addressed the responses of wetland plants to elevated CO_2, and still fewer have considered interactions between elevated CO_2 and other global change variables. In a field study of 'dry' tussock tundra (a weak net emitter of CH_4), elevated CO_2 did not have a sustained impact on photosynthesis unless a 4°C increase in air temperature also occurred (Tissue and Oechel 1987, Oechel et al. 1994). The authors suggested that warming relieved a severe nutrient limitation on photosynthesis by increasing N mineralization, illustrating an important interaction between elevated CO_2, nutrient availability, and temperature. In another example, the response of *Oryza* spp. (rice) to elevated CO_2 depended on N availability (Ziska et al. 1996).

Wetland plant responses to elevated CO_2 may be influenced by changes in patterns of flooding and soil saturation that are expected to accompany climate change (Cubasch et al. 2001, Poff et al. 2002). Flooding dramatically reduces plant growth, particularly of trees and other woody species. Flood-induced anoxia interferes with root respiration and the ability of plants to produce or maintain root biomass. Microbial respiration in the absence of O_2 produces end products such as Fe(II) and H_2S that have the potential to be

toxic or interfere with nitrogen assimilation (Tanaka et al. 1966, Koch et al. 1990). Hydrophytic plants reverse these effects to some extent by providing an internal path for O_2 flow to roots and through a variety of other physiological mechanisms (Crawford and Braendle 1996, Kozlowksi and Pallardy 2002). Despite such adaptations, woody plant production is almost always greater in partially unsaturated soils than flooded soils (Conner and Day 1976, Kozlowski 1984, Megonigal et al. 1997). This is not necessarily the case for herbaceous wetland species (Blanch et al. 1999, Lenssen et al. 1999), the most flood-tolerant of which have their greatest production when flooded (Kirkman and Sharitz 1993).

We know of no previous study that has investigated the effects of elevated CO_2 on a wetland tree, and there are no studies of the interaction between flooding and elevated CO_2 on any wetland plant species. Previous elevated CO_2 studies on temperate and tropical hydrophytic plant species have all been performed under flooded or saturated soil conditions (Curtis et al. 1989, Drake et al. 1996a, Baker et al. 1997, Megonigal and Schlesinger 1997, Koizumi et al. 2001), and all reported enhanced photosynthesis when it was measured. However, CO_2-enhanced photosynthesis will not necessarily translate into enhanced primary productivity because growth can be limited by nutrients, temperature, or other factors. Provided that growth is not severely limited by other factors (Tissue and Oechel 1987, Oren et al. 2001a), a CO_2-induced increase in photosynthesis may increase wetland net primary production by enhancing physiological or morphological adjustments to flooding stress. However, if flooding stress is severe, an increase in the photosynthate supply may not overcome limitations on root respiration imposed by O_2 availability, leading to no change in net primary production. Because trees are particularly sensitive to flooding, the interaction between flooding and elevated CO_2 can be expected to be stronger for trees than herbaceous plants.

The objectives of this study were to determine the physiological and growth responses of a wetland tree to elevated CO_2 and to compare the elevated-CO_2 responses of a tree species to an herbaceous species under flooded and non-flooded conditions. We tested three hypotheses: (1) elevated CO_2 will increase photosynthesis in both plant species regardless of the flooding treatment, (2) elevated CO_2 will increase the growth of the tree in the absence of flooding but not in the presence of flooding, and (3) elevated CO_2 will increase the growth of the herbaceous species irrespective of the flooding treatment.

MATERIALS AND METHODS

We studied the effects of elevated CO_2 and flooding on an emergent aquatic macrophyte, *Orontium aquaticum* L., and a woody conifer, *Taxodium distichum* (L.) Richard, that co-occur in the southeastern United States. *Orontium aquaticum* has better developed aerenchyma tissue and occurs on wetter sites than *T. distichum*, both of which suggest that the herbaceous species is more flood tolerant than the tree.

Experimental Procedure

Taxodium distichum seeds were obtained from a commercial source (F.W. Schumacher Co., Inc., Sandwich, MA); the seed tree was from the U.S. Gulf States region. *Orontium aquaticum* seeds were collected from a tidal freshwater wetland on the White Oak River, North Carolina (Megonigal 1996). Seeds of *O. aquaticum* were surface sterilized with a 1:10 bleach solution to prevent fungal growth and stored at 5°C until planting. *Taxodium distichum* seeds were stored for 6 days at 5°C under dry conditions, then soaked for 48 hours in 0.01% nitric acid to break dormancy. Seeds of both species were planted into flats filled with Canadian *Sphagnum* peat moss amended with dolomitic lime to raise the pH to approximately 6.7. Peat moss was used because it is harvested from organic wetland soils that are similar to the histosols on which our experimental species can occur naturally in southeastern North America (Megonigal 1996). Germination flats were placed into separate environmentally controlled growth chambers in which CO_2 concentrations were maintained near 350 or 700 ppm. Temperatures were 25/20°C (day/night) and photon flux density was 1000 $\mu mol\ m^{-2}\ s^{-1}$ during a 14-hour day-length period. Relative humidity in both chambers was >70%. Soils with *O. aquaticum* were watered until saturated and those with *T. distichum* seeds were maintained in a moist, but non-flooded, condition. Plants of approximately equal size with two true leaves (*O. aquaticum*) or six true leaves (*T. distichum*) were selected for the study and transplanted into 43-cm deep × 10-cm diameter polyvinyl chloride (PVC) containers. Two transplanted seedlings that died within one week were replaced with two seedlings of similar size.

Each PVC container had a ring of four 1-cm-diameter holes at 6 cm and 28 cm above the base of the container to allow for water exchange; a strip of aluminum screen was secured over the holes to prevent excessive soil loss. The bases were sealed with a 10-cm-diameter PVC end-cap and filled with 250 ml of pea gravel to prevent the pot from floating. Containers were filled to a depth of 39 cm with a peat-based soil that had been mixed three weeks before transplanting. The soil mixture consisted of 95% Canadian *Sphagnum* peat, 3.5% wetland soil collected from the White Oak River, North Carolina, and 1.5% dolomitic lime to raise the soil pH to 6.7. A slow-release all-purpose

fertilizer (Scott's Master Collection 15–13–13 NPK) was added to the soil at a rate of 3 ml L^{-1} soil. This was supplemented with a water-soluble fertilizer (Miracle-Gro 15–30–15 NPK) applied every three weeks at half strength beginning on 20 June.

Transplanted seedlings were grown in four environmentally controlled glasshouses at the Duke University Phytotron (Durham, NC, USA) from 9 June to 16 September. Atmospheric CO_2 concentrations were maintained at 350 or 700 ppm for each replicate (n=2) glasshouse. The glasshouse temperatures were controlled near 28°C (range 26.8 to 29.6°C) from 0600 to 2000 hours and 23°C overnight. Daily relative humidity levels were 60 to 70%. Filtered sunlight provided a photon flux density of about 1500 μmol m^{-2} s^{-1} at 1300 hours.

Eight *T. distichum* seedlings, 12 *O. aquaticum* seedlings, and 8 no-plant controls were randomly assigned to each combination of treatments (i.e. two levels of flooding × two levels of CO_2 concentration × two replicate glasshouses). Plants were placed into four large tubs in each glasshouse (two tubs per species), which allowed for different water treatments (flooded or non-flooded with tap water). Styrofoam disks (0.6-cm thick) were placed on the surface of the water to minimize algal growth and evaporation. Water levels for *O. aquaticum* were initially set at −3 cm (below the soil surface) on 10 June. As the seedlings grew, water levels were slowly changed over a 16-day period to −6 cm for the non-flooded treatment and +5 cm for the flooded treatment. *Taxodium distichum* water levels were initially set at −6 cm for the non-flooded and −2 cm for the flooded water treatments, then changed to the target levels of −10 cm and +5 cm. Water levels were maintained at the target levels by replacing evapotranspiration losses with tap water on a daily basis. Water was drained from the tubs and replaced every three weeks to prevent salt accumulation.

Photosynthesis

In situ photosynthetic measurements were made on a subset of six replicate plants per flooding and CO_2 treatment (i.e., pooled across replicate glasshouses) using a LICOR model 6200 portable photosynthetic system (Lincoln, NE) equipped with a 0.25-L leaf chamber. Measurements were made in the glasshouse between 0930 and 1500 hours at ambient light levels (1300 to 1500 μmol m^{-2} s^{-1}), which were saturating light levels for these species. At saturating light levels, the photosynthetic rates were fairly constant during the measurement period until about 1600 hours (data not shown).

Because photosynthetic rates often vary dramatically with leaf age, we measured a wide range of leaf ages in order to estimate whole-plant photosynthesis. *Orontium aquaticum* leaves were grouped into 11 discrete age classes subjectively judged to have similar photosynthetic rates. The oldest leaves in each class were 1, 2, 4, 5, 7, 10, 12, 14, 23, 41, and 78 d. *Taxodium distichum* leaves were grouped into new (within three branches of the apex, <1 week old) and old (within three branches of the base, ~18 weeks old). Photosynthesis measurements were made between 11 July and 15 September. Leaf area was determined by tracing the portion of the leaf enclosed by the LICOR 6200 cuvette onto paper, then passing it through a LI-COR 3100 leaf area meter. The area of the paper translated directly to leaf area for *O. aquaticum* which has broad, entire leaves. For *T. distichum* leaves, the paper area was divided by two to correct for spaces between the individual needles on the leaf. A comparison of this procedure to direct measurement with the LICOR 3100 leaf area meter showed a maximum 3% error between the two measurement methods. Total leaf area per age class was measured with a LICOR 3100 leaf area meter at the time of harvest and used to calculate whole-plant photosynthesis as follows:

$$\text{Whole-plant photosynthesis} = \sum LA_i \times A_i \quad (1)$$

where LA_i is the total area of leaves in age class *i* and A_i is the age-specific photosynthetic rate.

Biomass

Total plant biomass was determined after 18 weeks by destructively harvesting all plants. Roots were gently washed of soil and separated into fine (≤0.1 cm) and coarse (>0.1 cm) size classes. Plant material was dried for 5 days at 70°C.

Unlike *T. distichum*, *O. aquaticum* stems and foliage have a life span that is far shorter than the length of this study. To avoid missing a substantial portion of the *O. aquaticum* primary production, individual leaves were tagged and monitored daily for senescence. A shoot was considered new when the leaf was 25% open and senesced if >50% of the leaf area was yellow. Shoot size was measured at two-week intervals. Upon senescence, the shoot was removed from the plant and dried at 70°C for five days. Shoot production for the experimental period was calculated by adding the mass of senesced shoots to the biomass at the time of harvest. Tagging individual leaves also permitted us to calculate changes in leaf lifespan and turnover time, which influence primary production.

Whole-Plant Transpiration

Although our hypotheses do not directly concern transpiration responses, we measured transpiration

rates because they can indirectly influence plant growth through changes in soil saturation. Transpiration measurements were conducted on six replicate plants and six no-plant controls (i.e. soil only) in each of the four combinations of elevated CO_2 and water-table depth. PVC pots were placed into larger PVC pipes (53 cm $\times$ 15 cm diameter) that were sealed on the bottom. The larger pipes were filled with tap water to a designated water level. The surface of the soil and water was covered with Styrofoam peanuts and a piece of black plastic to minimize evaporation. Evapotranspiration was determined by measuring the rate of water-table recession over a period of three to five days. Transpiration rate was calculated as the difference between evapotranspiration from the planted containers and evaporation from the controls. On the last day of the measurement period, individual plant leaves were traced onto white paper and leaf area was determined with a leaf area meter as described above for determinations of photosynthesis.

Soil Redox Potential

In order to verify the effects of the flooding treatment on soil O_2 availability and reduction status, soil reduction-oxidation (redox) potential was measured at the end of the study using an Orion Model 250A redox meter at a depth 6.5 cm below the soil surface. Values represent redox under standard conditions relative to a H_2 electrode.

Statistical Analysis

The SAS univariate procedure was used to assess normality (SAS Institute 1987) and Levene's test for equality of variance was used to assess homoscedasticity. Data were log-transformed when non-normal and were analyzed for statistical differences between the main effects (flooding and CO_2 treatment) by two-way ANOVA using the SAS GLM procedure. CO_2 effects were calculated using a Type III mean-square-error term with glasshouse nested within the CO_2 treatment. Similarly, other Type III mean-square-error terms in the same model were used to test for the effects of flooding, the interaction between the flooding and CO_2 treatments, and significant differences between replicate glasshouses as appropriate (e.g., the Type III term for the flooding treatment was flooding crossed with glasshouse nested within the CO_2 treatment). When significant interactions between CO_2 and flooding occurred, the effects were split and analyzed using a t-test. In order to simplify the data presentation, the means and errors reported in all tables and graphs were calculated by pooling data across the two replicate glasshouses (i.e., n=16–20 for a given combination of CO_2 level and flooding level). However, all of the statistical outcomes reported in the text, figures, and tables are based on the ANOVA tests described above. We set statistical significance at $\alpha=0.10$ to reduce the probability of a type II error from the low power (n=2) of the experimental design.

RESULTS

Soil Redox Potential

Flooding significantly reduced the soil redox potential for both *T. distichum* ($P=0.0006$, $F_{1,2}=1720$) and *O. aquaticum* ($P=0.0002$, $F_{1,2}=6019$) (Table 1). Due to the absence of radial O_2 loss from the roots, the no-plant control pots had more reduced soils in both flooding treatments when compared with planted pots (t-test, $P<0.0001$, $df>90$, Table 1). Soil redox potential was not affected by elevated CO_2. There was a curious and unexplained interaction between elevated CO_2 and flooding in the no-plant control ($P=0.008$, $F_{1,2}=130$), in which elevated CO_2 caused a significant decrease in redox potential in the non-flooded treatment only.

Biomass

There was no effect of elevated CO_2 on *T. distichum* shoot biomass or total biomass in either water-table treatment. However, there was a significant interaction between CO_2 treatment and water table in total *T. distichum* root mass ($P=0.067$, $F_{1,2}=14$). Elevated CO_2 had no effect in the flooded treatment but caused an increase in total root biomass in the non-flooded treatment ($P=0.078$, t-test). In the non-flooded treatment there was also a trend for elevated CO_2 to increase *T. distichum* biomass by 32–49% in every aboveground tissue subcategory (Table 1), but none of the differences were significant. The only elevated CO_2 effect on *T. distichum* biomass in the flooded treatment was a decrease in fine root mass ($P=0.056$, t-test), which we cannot fully explain. Flooding significantly reduced total *T. distichum* biomass by 54–81% ($P=0.007$, $F_{1,2}=139$).

Elevated CO_2 increased *O. aquaticum* biomass by 17–55% regardless of the flooding treatment, and this effect was observed in all biomass subcategories (Table 1), including total shoot biomass ($P=0.027$, $F_{1,2}=35$), total root biomass ($P=0.028$, $F_{1,2}=34$), and total biomass ($P=0.024$, $F_{1,2}=40$). Elevated CO_2 had no effect on leaf lifespan. In contrast to its effects on *T. distichum*, flooding increased *O. aquaticum* biomass by 29–57% in all subcategories, including total shoot biomass ($P=0.022$, $F_{1,2}=44$), total root biomass ($P=0.069$, $F_{1,2}=13$), and total biomass ($P=0.041$,

Table 1. Biomass, productivity and other growth parameters for *Taxodium distichum* and *Orontium aquaticum* plants after 18 weeks at ambient or elevated CO_2. Values are expressed as mean (SD) for data pooled across replicate glasshouses. Statistical inferences are based on ANOVA tests on non-pooled data.

Parameters	Flooded Treatment		Non-flooded Treatment		Significant Difference†
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2	
Taxodium distichum					
Total Shoot Mass (g)	6.57 (2.6)	5.78 (1.4)	17.9 (5.3)	24.5 (12)	W
Fine Root Mass (g)	0.403 (0.21)	0.298 (0.17)	1.18 (0.38)	1.55 (0.69)	C_f WI
Coarse Root Mass (g)	0.750 (0.36)	0.683 (0.32)	1.62 (0.49)	2.41 (1.4)	W
Total Root Mass (g)	1.15 (0.56)	0.980 (0.46)	2.80 (0.78)	3.97 (1.9)	C_{nf} WI
Total Biomass (g)	7.72 (3.1)	6.76 (1.8)	20.7 (6.0)	28.4 (14)	W
Leaf Area (cm^2)	370.5 (116)	260.9 (89)	1631 (618)	1518 (620)	W
Leaf-Level Photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)	7.68 (2.1)	19.7 (3.8)	9.20 (2.0)	20.8 (6.2)	CW
Whole-Plant Photosynthesis ($\mu mol\ s^{-1}$)	0.300 (0.095)	0.505 (0.18)	1.57 (0.59)	3.32 (1.2)	CW
Soil Redox Potential (mV)	−260 (25)	−283 (18)	151 (13)	136 (12)	W
Orontium aquaticum					
Total Shoot Mass (g)	7.87 (2.2)	10.3 (3.2)	5.97 (1.9)	7.21 (2.4)	CW
Fine Root Mass (g)	1.51 (0.51)	2.14 (0.78)	0.956 (0.48)	1.47 (0.71)	CW
Coarse Root Mass (g)	5.49 (1.35)	7.38 (2.4)	3.71 (1.3)	5.74 (2.0)	CW
Total Root Mass (g)	6.99 (1.7)	9.52 (2.9)	4.66 (1.6)	7.21 (2.5)	CW
Total Biomass (g)	14.3 (3.4)	19.1 (5.7)	10.3 (3.4)	13.9 (4.4)	CW
Leaf Lifespan (d)	50.9 (9.4)	46.3 (11)	48.7 (12)	47.6 (9.7)	N
Leaf Area (cm^2)	723.9 (172)	644.4 (162)	542.4 (173)	483.3 (185)	W
Leaf-level Photosynthesis‡ ($\mu mol\ m^{-2}\ s^{-1}$)	11.0 (5.3)	22.0 (7.3)	11.1 (4.9)	26.6 (9.4)	C
Whole-plant Photosynthesis ($\mu mol\ s^{-1}$)	0.711 (0.19)	1.23 (0.34)	0.549 (0.181)	1.08 (0.43)	CW
Soil Redox Potential (mV)	−225 (11)	−238 (12)	143 (16)	144 (17)	W
No-Plant Control					
Soil Redox Potential (mV)	−297 (13)	−297 (11)	95.2 (5.9)	89.8 (5.5)	C_{nf}WI

† Significant differences indicated by the letters C, W, I or N (C = significant CO_2 effect, C_f = significant CO_2 effect in the flooded treatment only, C_{nf} = significant CO_2 effect in the non-flooded treatment only, W = significant water table treatment, I = significant interaction between CO_2 and water table treatment, and N = no significant effect). The significance threshold was set at $\alpha = 0.10$.
‡ Leaf-level photosynthesis means for *O. aquaticum* exclude leaves ≤ 1 d old.

$F_{1,2}=23$) (Table 1). Flooding had no effect upon leaf lifespan. There was no significant interaction between CO_2 treatment and flooding in *O. aquaticum* biomass.

Leaf-Level Photosynthesis

Elevated CO_2 increased *T. distichum* ($P=0.006$, $F_{1,2}=164$) and *O. aquaticum* ($P=0.049$, $F_{1,2}=19$) leaf-level photosynthesis in both the flooded and non-flooded treatments. Flooding decreased leaf-level photosynthesis in *T. distichum* ($P=0.096$, $F_{1,2}=9$) but not in *O. aquaticum*. In both species, leaf-level photosynthesis was not significantly affected by the interaction between CO_2 treatment and flooding.

Orontium aquaticum photosynthesis at ambient CO_2 increased as the leaves aged from 1 to 5 days, then decreased significantly thereafter ($r^2=0.43$, $P<0.001$, $F_{1,56}=42$, Figure 1). The CO_2-enriched leaves also experienced a significant reduction in leaf-level photosynthesis following the peak ($r^2=0.53$, $P<0.001$, $F_{1,28}=38$, Figure 1). Linear regressions indicate that photosynthetic rates for *O. aquaticum* decreased with time more rapidly at elevated CO_2 than at ambient CO_2, although elevated CO_2 delayed the decrease in photosynthesis by about 7 days (Figure 1). For both species, there was no correlation between leaf-level photosynthesis and time since the start of the experiment to indicate that the effect of elevated CO_2 on photosynthesis decreased over time (data not shown, Vann 2000).

Whole-Plant Photosynthesis

Whole-plant photosynthesis was calculated as the product of leaf area and leaf-level photosynthesis (equation 1). *Taxodium distichum* leaf area was sig-

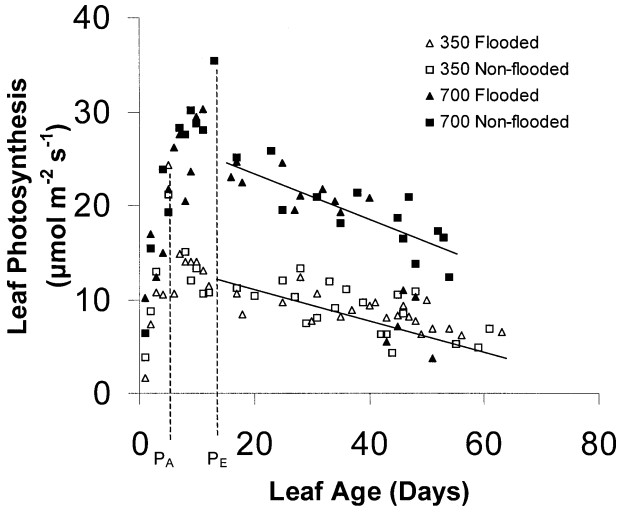

Figure 1. Leaf photosynthesis per age class of *Orontium aquaticum*. Vertical dashed lines indicate the single sample where peak rates of photosynthesis occurred in the ambient (P_A) and elevated (P_E) CO_2 treatments. Linear regressions indicated that photosynthetic rates declined with time more rapidly at elevated CO_2 ($y = -0.356X + 30.9$, $r^2 = 0.54$) than ambient CO_2 ($y = -0.116X + 13.2$, $r^2 = 0.43$).

nificantly reduced in the flooded treatment ($P=0.010$, $F_{1,2}=94$) but was not affected by elevated CO_2 (Table 1). Elevated CO_2 enhanced *T. distichum* whole-plant photosynthesis in both the non-flooded and flooded treatments ($P=0.066$, $F_{1,2}=14$, Table 1). At elevated CO_2, *T. distichum* whole-plant photosynthesis increased 69% in the flooded treatment and 112% in the non-flooded treatment. Flooding alone reduced *T. distichum* whole-plant photosynthesis by 81–85% ($P=0.007$, $F_{1,2}=147$) (Table 1). There was no significant interaction between CO_2 treatment and flooding for leaf area or whole-plant photosynthesis in *T. distichum*.

Leaf area of *O. aquaticum* was significantly greater in the flooded treatment ($P=0.010$, $F_{1,2}=95$) but was unaffected by elevated CO_2. Elevated CO_2 increased *O. aquaticum* whole-plant photosynthesis 73% to 97% ($P=0.004$, $F_{1,2}=280$, Table 1). Flooding alone also increased *O. aquaticum* whole-plant photosynthetic rates ($P=0.05$, $F_{1,2}=18$). There was no significant interaction between CO_2 treatment and flooding for leaf area or whole-plant photosynthesis in *O. aquaticum*.

Transpiration

Elevated CO_2 decreased rates of whole-plant transpiration in both *T. distichum* and *O. aquaticum*, but the difference was statistically significant for *O. aquaticum* only ($P=0.020$, $F_{1,2}=49$, Figure 2). Flooding caused a large decrease in transpiration in *T. distichum* ($P=0.003$, $F_{1,2}=295$). There was a significant interaction between flooding and CO_2 treatment in *O. aquaticum* ($F=0.005$, $F_{1,2}=206$) where flooding had no effect on transpiration at ambient CO_2 but caused an increase in transpiration at elevated CO_2.

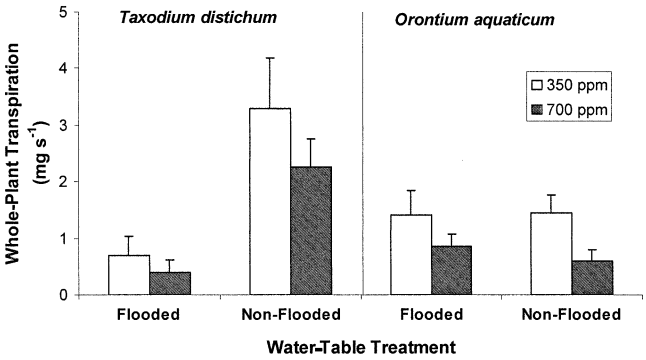

Figure 2. Whole-plant transpiration of *T. distichum* and *O. aquaticum* at 18 weeks of treatment. Values are means ±1 standard deviation pooled across replicate glasshouses (i.e., n=16 for *T. distichum*, n=24 for *O. aquaticum*).

Glasshouse Effects

There was a significant difference between replicate glasshouses for all *T. distichum* variables except transpiration and leaf-level photosynthesis. For *O. aquaticum*, the only variables for which replicate glasshouses were significantly different from each other were leaf-level photosynthesis and soil redox potential.

DISCUSSION

Our results provide support for the hypothesis that flooding suppresses the growth response of flood-tolerant trees such as *T. distichum* to elevated CO_2. Elevated CO_2 increased *T. distichum* total root biomass in the non-flooded treatment, but there was no CO_2 simulation of biomass in the flooded treatment. By comparison, elevated CO_2 increased the growth of *O. aquaticum* in all biomass categories regardless of the flooding treatment. The results demonstrate that plant growth responses to elevated CO_2 can interact with flooding in wetland ecosystems. Furthermore, the extent of the interaction will vary by plant species and perhaps by plant growth form (i.e., woody versus herbaceous aquatic macrophyte).

Flooding limited the growth response of *T. distichum* to elevated CO_2 despite a sustained increase in photosynthesis, suggesting that growth was limited by some factor other than the CO_2 assimilation rate. At ambient CO_2, flooding reduced *T. distichum* total root biomass by 59%, but leaf-level photosynthesis was reduced by just 17% (Table 1). A flood-induced reduction in growth, but not photosynthesis, was reported

in a previous study of *T. distichum* (Pezeshki et al. 1996). These results indicate that the physiological limitations to growth imposed by flooding at ambient CO_2 were not ameliorated by the enhanced supply of photosynthate at elevated CO_2.

One possible reason that *T. distichum* did not show a growth response to elevated CO_2 in the flooded treatment is nutrient limitation. Even though the plants were fertilized with N, P, and K, flooding could have conceivably interfered with nutrient uptake or metabolism (e.g., Rosen and Carlson 1984). However, photosynthetic rates of *T. distichum* at elevated CO_2 did not decrease over the course of the study ($r^2<0.15$, $P=0.76$, $n=5$), and nutrient limitation would have caused photosynthetic rates in plants grown at elevated CO_2 to decrease over time (Drake et al. 1996a, Oren et al. 2001a). This is consistent with reports that N metabolism is not necessarily sensitive to flooding in freshwater flood-tolerant trees (Pezeshki et al. 1999, Kreuzwieser et al. 2002). Thus, it is unlikely that the lack of a *T. distichum* growth response to elevated CO_2 under flooded conditions was due to nutrient limitation.

Since the photosynthate subsidy afforded to *T. distichum* grown in elevated CO_2 was not converted to biomass, it must have been consumed by other physiological processes. A possible sink for the excess photosynthate is respiration. There are respiration costs for physiological adaptations such as anaerobic enzyme production (Vartapetian and Jackson 1997) and morphological adjustments such as development of aerenchyma tissue and specialized roots (Megonigal and Day 1992). Root system morphology was distinctly different for flooded and non-flooded *T. distichum* seedlings after 18 weeks (C. Vann, pers. obs.), indicating that such adjustments had been made. Respiration efficiency may have decreased because the roots were partially dependent on fermentation respiration, which yields just 6% of the ATP from glucose of aerobic respiration (Boamfa et al. 2003). Anoxia may have caused lingering damage to respiratory pathways (Crawford and Braendle 1996, Boamfa et al. 2003), mediated in part by soil toxins such as ferrous iron (Snowden and Wheeler 1993). The collective effect of these processes may have increased root respiration rates, thereby offsetting the increase in photosynthate supply and preventing a CO_2 stimulation of shoot growth.

Elevated CO_2 increased *O. aquaticum* total root biomass by 36–55% compared to ambient CO_2 plants, regardless of the flooding treatment. For *T. distichum*, elevated CO_2 increased total root biomass by 42% in the non-flooded treatment but slightly decreased root biomass by 15% in the flooded treatment. This difference probably reflects the fact that *O. aquaticum* occurs on sites that are too wet to support *T. distichum*. It is likely that the different elevated CO_2 responses shown by these two species applies more generally to woody and herbaceous wetland plants, as reflected by the presence of woody species in drier positions along flooding gradients (Mitsch and Gosselink 1993). Indeed, flooding has either a neutral or negative effect on tree productivity (Megonigal et al. 1997) but can stimulate herbaceous aquatic macrophyte productivity (Kirkman and Sharitz 1993, Blanch et al. 1999).

Transpiration

Elevated CO_2 may also influence plant growth indirectly by altering the saturation status of soils via transpiration rates (Megonigal and Schlesinger 1997, Jackson et al. 1998). We previously reported that elevated CO_2 decreased *O. aquaticum* transpiration on a leaf-area basis (Megonigal and Schlesinger 1997). In the present study, elevated CO_2 decreased *O. aquaticum* transpiration by 42–66% on a whole-plant basis. It is unclear whether these effects will hold when scaled to intact plant canopies. In studies of upland systems, elevated CO_2 has decreased evapotranspiration in some cases (Hungate et al. 2002) and had little effect in other cases (Pataki et al. 1998, Wullschleger and Norby 2001). *Taxodium distichum* forests may respond differently than species that have been considered to date because they grow in soils that are often saturated (e.g., Oren et al. 1999, Oren et al. 2001b).

SUMMARY AND IMPLICATIONS

Elevated CO_2 increased the growth of a hydrophytic tree (*T. distichum*) when the water-table depth was below the soil surface but not when it was flooded. By comparison, elevated CO_2 increased the growth of an herbaceous emergent aquatic macrophyte (*O. aquaticum*), which is less sensitive to flooding than tree seedlings, irrespective of water-table position. These results suggest that flooding has the potential to limit plant growth responses to elevated CO_2 in wetlands dominated by woody species. If so, swamp forests will be among the most sensitive ecosystem types to interactions between elevated CO_2 and flooding.

Caution should be exercised when extrapolating the data presented here because flooding affects *T. distichum* seedlings, saplings, and mature trees differently (Megonigal and Day 1992), and seedlings are the most flood sensitive developmental stage. Older *T. distichum* trees with root systems adapted to flooding may have been able to allocate more of the photosynthate subsidy from elevated CO_2 into stem growth. Nonetheless, the results suggest that the potential for elevated CO_2 to enhance carbon sequestration in wood is

greater in dry-end wetland forests than in wet-end wetland forests.

It is important to determine the extent to which changes in carbon sequestration and CH_4 emissions will offset one another in terms of radiative climate forcing (i.e., changes in the energy balance of the atmosphere that drive global warming). Elevated CO_2 stimulated CH_4 emissions in the same system as the present study by up to 69% (Vann and Megonigal 2003). The present study was an initial test of hypotheses concerning the nature of interactions between elevated CO_2 and flooding on plant growth, which will influence the ability of wetland forests to sequester carbon in biomass. The results argue for a larger-scale test of these hypotheses, preferably in the field.

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